



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Lloyd Wolfinbarger, Jr.

Appl. No.:08/619,412

Filed: March 21, 1996

For: **Process for Cleaning Large Bone
Grafts and Bone Grafts Produced Thereby**

Art Unit: 3306

Examiner: Nguyen, A. 

Atty. Docket: LNR.F.006

DECLARATION UNDER RULE 1.131(a)

Commissioner of Patents and Trademarks
Washington, D.C.20231

Dear Sir:

Dr. Lloyd Wolfinbarger, Jr., Phd., the applicant in the above-identified patent application, and Billy G. Anderson, president and CEO of LifeNet Research Foundation, the assignee, declare as follows:

1. That sometime prior to January 21, 1994, Dr. Lloyd Wolfinbarger, a research scientist, conceived of a method for cleaning intact bone grafts using negative or vacuum pressure to remove bone marrow and bone marrow elements from a bone graft;
2. That said method included subjecting the essentially intact bone graft including the internal matrix thereof to a vacuum mediated flow of solvent to remove bone marrow elements to produce a cell-free bone matrix;
3. That Dr. Lloyd Wolfinbarger successfully performed the method prior to January 21, 1994, as evidenced by Exhibit A attached hereto. Exhibit A includes (i) a


copy of Dr. Wolfinbarger's dated (October 1, 1992) notebook page illustrating the method for cleaning bone using a vacuum mediated flow of solution; (ii) a copy of two pages dated March 1, 1993 showing the results obtained using the vacuum method to clean bone, the graph illustrating protein concentration versus detergent volume; and (iii) a copy of two photographs, front and back, dated "May 1993" on the back, illustrating bone being processed using the vacuum mediated method;

4. After successful performance of the method in 1993, LifeNet Research Foundation, the assignee of the present application optimized and perfected the method with Dr. Lloyd Wolfinbarger, and filed a patent application on February 27, 1995, issued on September 17, 1996 as U.S. patent no.: 5,556,379 (Exhibit B), which is the parent application of the present application, entitled: "Process for Cleaning Large Bone Grafts and Bone Grafts Produced Thereby" which is directed to cleaning bone using a vacuum mediated flow of solution;
5. After filing of the '379 patent application, Standard Operating Procedures (SOP's) were designed, as evidenced by Exhibit B attached hereto which includes a copy of two documents that are dated SOP's reflecting the vacuum mediated flow method of cleaning bone, that Billy G. Anderson President and CEO of LifeNet Research Foundation, on or about May 15, 1995, put into use as evidenced by Exhibit C attached hereto which states that the method was instituted on May 15, 1995;

6. That Billy G. Anderson President and CEO of LifeNet Research Foundation, on or about May 29, 1996 put the present negative pressure mediated recirculation method as recited in the SOP's attached hereto as Exhibit C into use as evidenced by Exhibit D which includes a copy of two documents which show the recirculation method and reflects an effective date of May 29, 1996 and an approval date of May 28, 1996 (document 163.002) and May 21, 1996 (document 034.005);

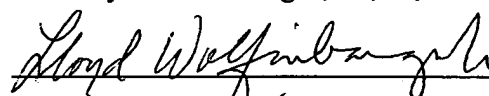
The declarants further state that the above statements were made with the knowledge that willful false statements and the like are punishable by fine and /or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statement may jeopardize the validity of this application or any patent resulting therefrom.

Mr. Billy G. Anderson, President and CEO



Date: 1-14-98

Dr. Lloyd Wolfinbarger, Jr., Phd.



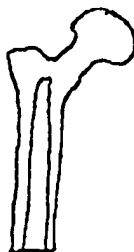
Date: 1/15/98

EXHIBIT A

Paraviride Processing of Bone

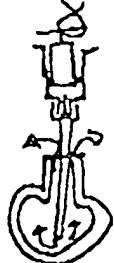
10/1/92

I Forms on femur heads, proximal femurs, distal femurs.



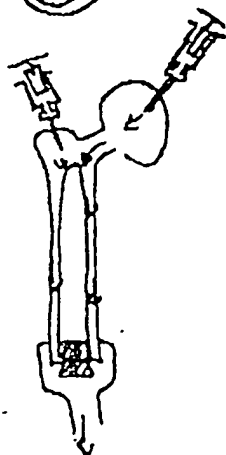
Post-It [®] brand fax transmittal memo 7671		# of pages	7
To	Bill	From	Shoyt
Co.		Co.	
Dept.		Phone #	
Fax #		Fax #	

II Flushing protocols



- 1) Flush @ varying volume of paraviride
- 2) Flush @ H_2O_2 (varying volumes)
- 3) Flush @ H_2O (varying volumes)
- 4) etc.

temp. ?
45°C



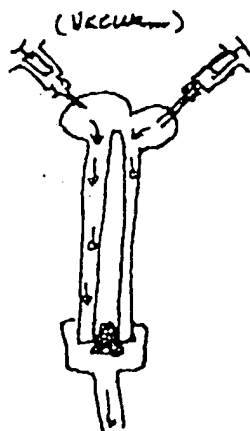
Objective:

— Use Paraviride to "solubilize" the bone marrow tissue; varying volumes may be necessary to effectively solubilize the bone marrow tissue in the cancellous bone areas

— Use hydrogen peroxide to oxidize remaining tissue debris and create a positive pressure behind the solubilized bone marrow tissue to "force" it out of the cancellous bone.

— Use water wash to flush paraviride & residual H_2O_2 from cancellous bone.

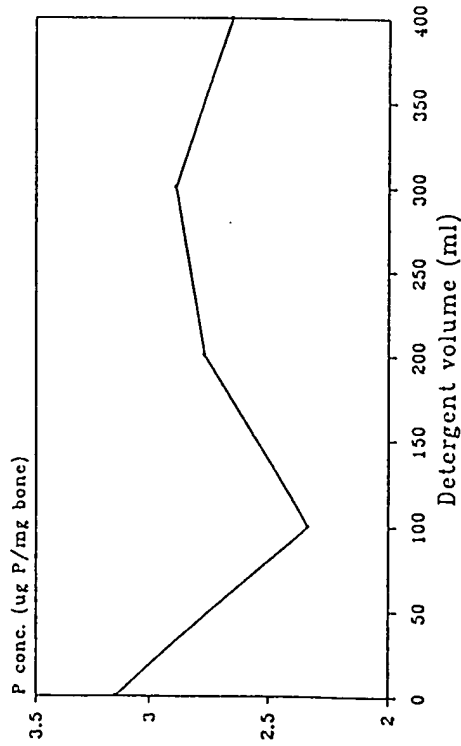
— Options: use other detergent solutions i.e. 2% sodium dodecyl sulfate (an anionic detergent) etc.



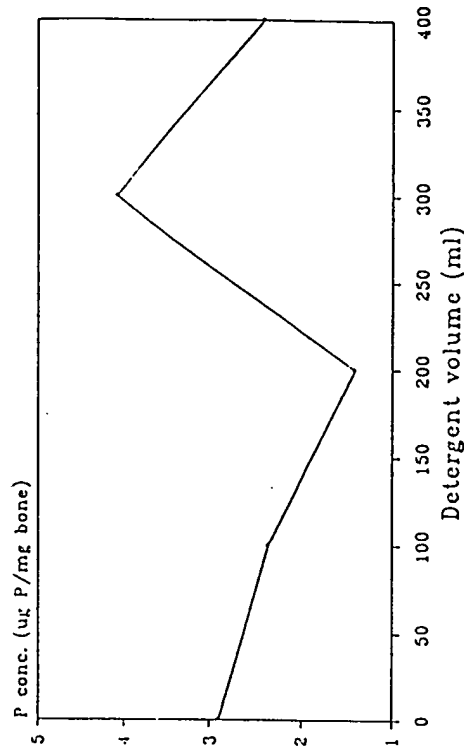
VACUUM

- Negative Pressure - Allowash™

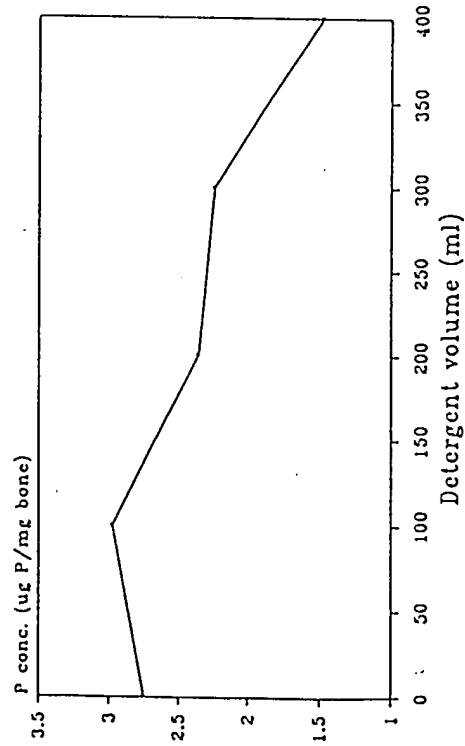
Position No.1 - 3/1/93 Flus
0.5 N NaOH solub., 37 deg., 17 hrs



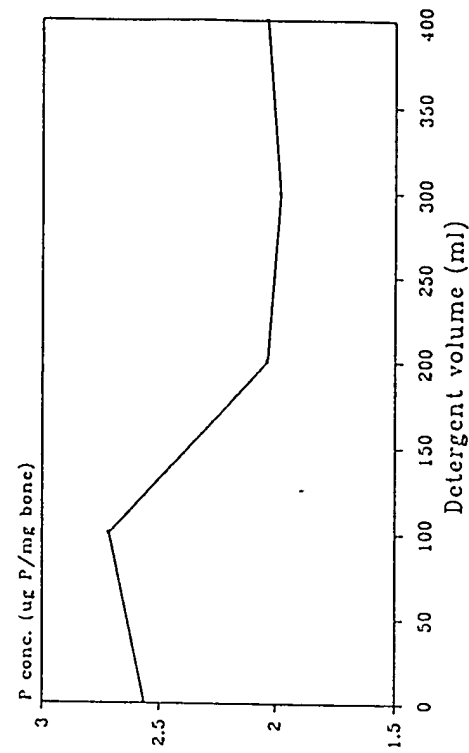
Position No.2 - 3/1/93 Flus
0.5 N NaOH solub., 37 deg., 17 hrs



Position No.3 - 3/1/93 Flus
0.5 N NaOH solub., 37 deg., 17 hrs



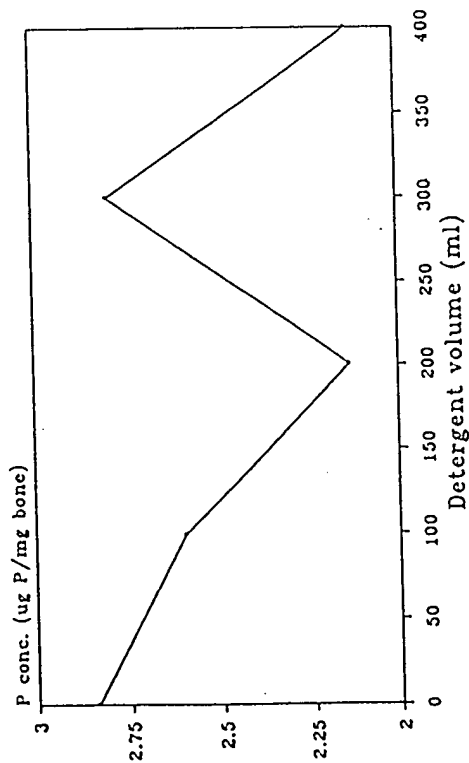
Position No.4 - 3/1/93 Flus
0.5 N NaOH solub., 37 deg., 17 hrs



Negative Pressure - Allowash™

LIFENET
5809 WARD COURT
VIRGINIA BEACH, VA 23455

AVERAGE- 3/1/93 Flushing
0.5 N NaOH solub., 37 deg., 17 hrs



AVERAGE- 3/1/93 Flushing
0.5 N NaOH solub., 37 deg., 17 hrs

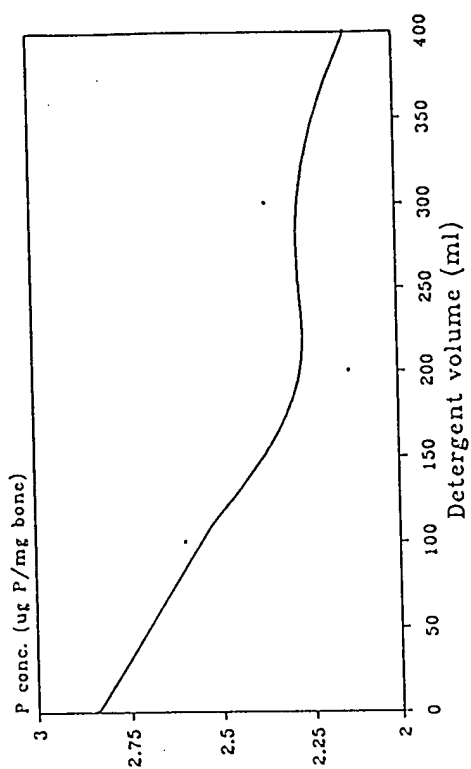


EXHIBIT B



Allowance ^{Tn} - Negative Reson

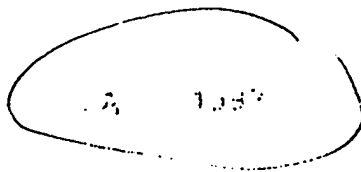
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AllowashTM — Negative Pres

LIFENET
5800 WARD COURT
VIRGINIA BEACH, VA 23455



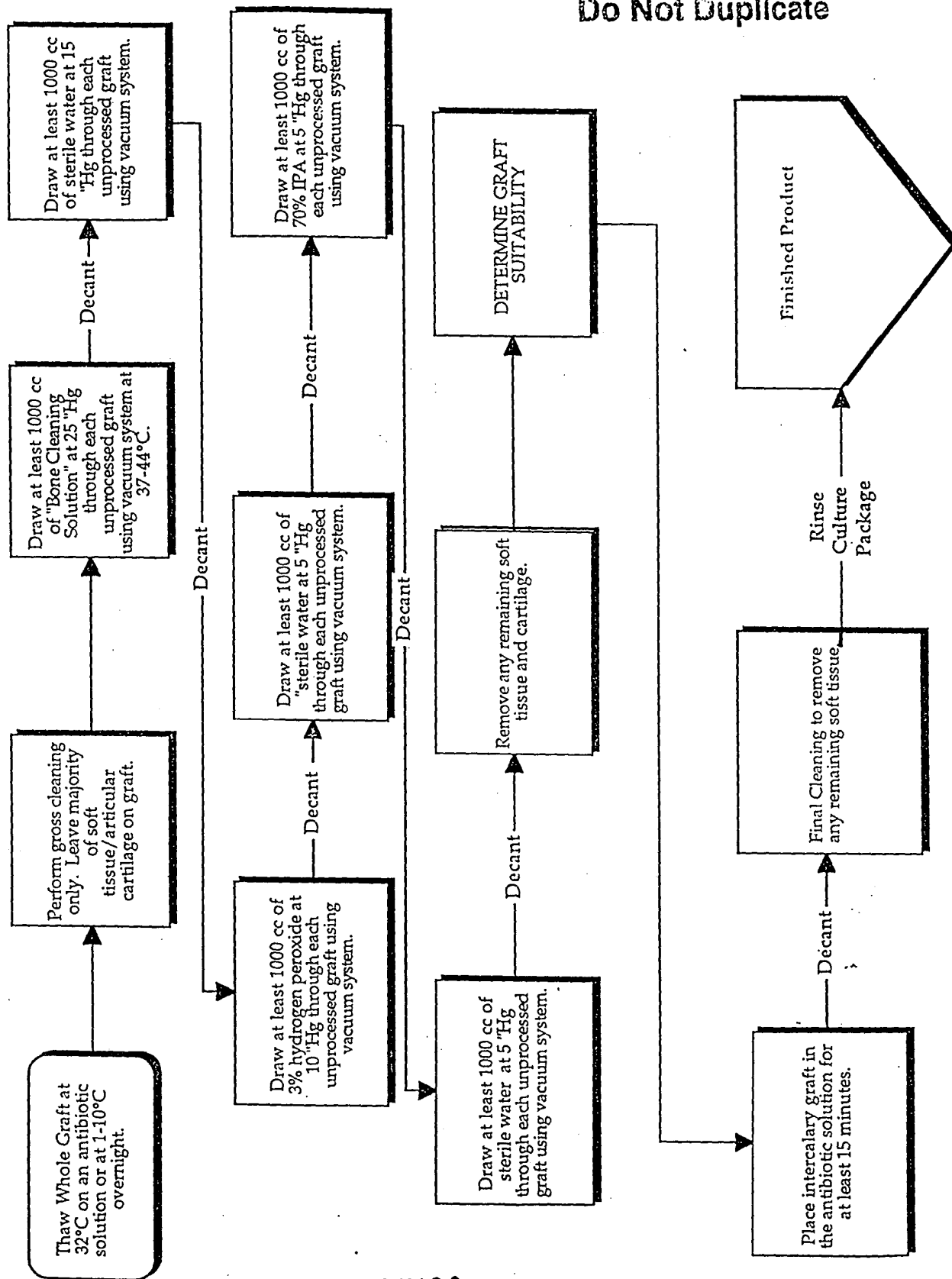
MAY 93

EXHIBIT C

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Best Available Copy

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Supersedes: PRO-MS-033.002	Written by: BB	Effective Date:
Dept. Approval:	Division Approval:	QA Approval:
Distribution	VP TP Svc	VP Tissue Svcs
	VP Corp Svcs	MS
	CV	SPD
	QA	PTB
	SS	
Annual QA Review:		

REFERENCES: MUSCULOSKELETAL TISSUE PROCESSING TRAYS, PRO-MS-060,
PRO-MS-064

PROCEDURE:

- A. Remove some of the soft tissue and periosteum from the graft material using sharp dissection techniques and periosteal elevators. DO NOT REMOVE THE SOFT TISSUE BETWEEN THE FEMORAL HEAD AND GREATER TROCHANTER. THE ENTIRE LIGAMENT MUST BE REMOVED FROM THE FEMORAL HEAD FOVEA.
- B. Transect the graft to no more than 22cm in length using a Stryker® saw or band saw. Ensure the cut is straight and contains no bone fragments. Remove all the muscle and periosteum approximately 2" proximal to the cut end.
- C. Remove the surface cartilage from the femoral head with either a scalpel blade, elevator, or osteotome.
- D. Using a 3/8" drill bit approximately 6" long, remove the contents of the intramedullary canal.
- E. Connect the intercalary vacuum apparatus to the cut end of the graft. Wrapping a cut end of the graft with a latex strip prior to attaching the intercalary vacuum apparatus may enhance the connection. Use only enough stripping to ensure a tight seal.

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- F. Connect an O-clamp to the intercalary apparatus/latex/ and graft junction and tighten. Connect a 1/2" vacuum line to one of the intercalary apparatus. To further enhance the connection, another latex strip and Parafilm® may be wrapped around the junction of the intercalary apparatus and graft.
- G. Connect the 1/2" vacuum tubing from the intercalary apparatus to a port on the sterile vacuum manifold. The vacuum manifold is attached to the collection canister at the "Ortho" port using 1/2" vacuum tubing. A 3/16" vacuum line is then attached to the vacuum pump from the collection canister.

Cap all unused ports on the vacuum manifold prior to turning the vacuum pump on.

- H. Place the graft and intercalary vacuum apparatus set-up in a deep sterile basin.
- I. Pour approximately 6000cc of a 1:100 dilution of Osteoclenz™ into the deep sterile basin. The Osteoclenz™ dilution is prepared by adding 6cc of 10x Osteoclenz™ to 5994 cc of sterile water. Ensure the basin is clearly labeled as "Osteoclenz™."

The temperature of the solution must be between 37° and 44° C. Place the deep sterile pan on a warmer if necessary. Each of the grafts must remain immersed in fluids at all times.

- J. Critical Step: Set the vacuum pressure to 25 in.Hg and draw the Osteoclenz™ through the graft(s). At least 1L of Osteoclenz™ must be infused through each allograft. Record the vacuum pressure, volume of infused solution and temperature of the Osteoclenz™ on the "Tissue Processing Log Worksheet."

Periodically inspect the vacuum lines. Any large bubbles indicate a leak in the vacuum tubing set-up. Tighten the O-Ring, reapply the latex stripping, or add Parafilm® to secure the seal.

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- K. Critical Step: Decant any remaining 1:100 dilution of Osteoclenz™ solution and add approximately 6L of sterile water to the deep sterile basin. Ensure the basin is clearly labeled as "Sterile Water." Draw at least 1L of water through the graft at 15 in.Hg. Record the vacuum pressure, volume of infused solution and exposure time on the "Tissue Processing Log Worksheet."
- L. Critical Step: Decant any remaining sterile water and add approximately 6L of 3% hydrogen peroxide to the deep sterile basin. Ensure the basin is clearly labeled as "Hydrogen Peroxide." Draw at least 1L of 3% hydrogen peroxide through the graft at 10 in.Hg. Record the vacuum pressure, volume of infused solution and exposure time on the "Tissue Processing Log Worksheet."
- M. Critical Step.: Decant any remaining 3% hydrogen peroxide and add approximately 6L of sterile water to the deep sterile basin. Ensure the basin is clearly labeled as "Sterile Water." Draw at least 1L of sterile water through the graft at 5 in.Hg. Record the vacuum pressure, volume of infused solution and exposure time on the "Tissue Processing Log Worksheet."
- N. Decant any remaining sterile water and add approximately 6L of 70% isopropyl alcohol to the deep sterile basin. Ensure the basin is clearly labeled as "70% IPA." Draw at least 1L of 70% isopropyl alcohol through the graft at 5 in.Hg. Record the vacuum pressure, volume of infused solution and exposure time on the "Tissue Processing Log Worksheet."
- O. Critical Step.: Decant any remaining 70% isopropyl alcohol and add 3L of sterile water to the deep sterile basin. Ensure the basin is clearly labeled as "Sterile Water." Draw at least 1L of sterile water through the graft at 5 in.Hg. Record the vacuum pressure, volume of infused solution and exposure time on the "Tissue Processing Log Worksheet."

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P. Remove the remaining soft tissue and cartilage and place the graft(s) in a basin with the antibiotic solution for at least 15 minutes. Ensure the basin is clearly labeled as "Antibiotics." Record the exposure time to the antibiotics on the "Tissue Processing Log Worksheet."

Q. Remove any remaining soft tissue if necessary and rinse again before culturing.

If the sterile wire wheel is used to remove the remaining soft tissue, ensure the Lucite™ capture box is surrounding the graft to minimize soft tissue discharge.

R. Assign the graft(s) the appropriate identification number, record the measurements on the processing log worksheet (record the condyle width, side, and total graft length), and culture the fashioned graft(s) for bacterial contamination with sterile cotton-tipped applicators. Place one cotton-tipped applicator into a TGC tube, and one into a TSB tube, label with the graft identification number. Refer to PRO-MS-060.

S. The graft material is now ready for packaging and placement in the appropriate freezer. Refer to PRO-MS-064. The graft must be x-rayed if requested.

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Allowash™ Treated Frozen Intercalary Tissue/Positive Pressure

- Frozen Proximal Tibia
- Frozen Proximal Humerus
- Frozen Proximal Femur with Head
- Frozen Proximal Femur
- Frozen Distal Femur

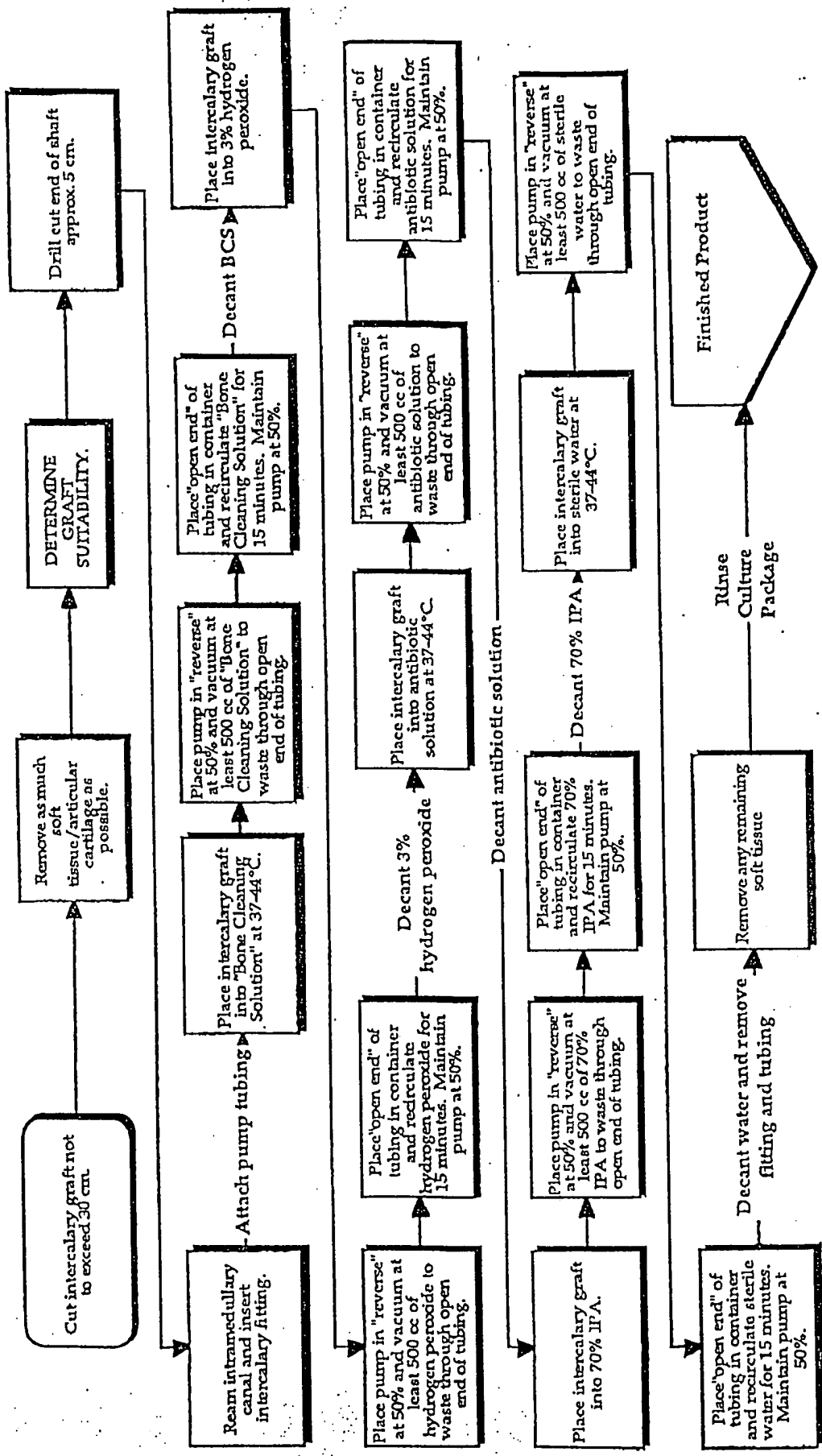
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EXHIBIT D

DOCUMENT#: PRO-MS-163.002
TITLE: ALLOWASH™ INTERCALARY GRAFT FLOW DIAGRAM

Supersedes: PRO-MS-163.001	Written by: BB	Effective Date: MAY 29 1996
Depl. Approval: <i>[Signature]</i> 5/28/96	Division Approval: <i>[Signature]</i> 5/28/96	QA Approval:
Distribution: VP TP Svcs VP Tissue Svcs VP Corp Svcs MS CV SPD QA SS	Annual QA Review:	

REFERENCES: PRO-MS-036, PRO-MS-035, PRO-MS-034, PRO-MS-033, PRO-MS-028



TITLE: PROCESSING FROM PROX. FEMORA WITH HEAD

Supercodes: PRO-MS-034.004	Written by: CKW,AH	Effective Date: May 29, 1996
Dept. Approval: ED 5/31/96	Division Approval: SS 5/23/96	QA Approval: LA 5/23/96
Distribution: VP TP Svc VP Tissue Svcs VP Corp Svcs MS CV SPD QA SS		
Annual QA Review:		

REFERENCES: PRO-MS-010, PRO-MS-060, PRO-MS-064, PRO-MS-136

PROCEDURE:

- A. Transect the graft to the desired length using a Stryker® saw or band saw. Ensure each bisected piece is not more than 30 cm in length. Ensure the cut is straight and contains no bone fragments.
- B. Remove all of the soft tissue and periosteum from the graft material using sharp dissection techniques and periosteal elevators.
- C. Remove the surface cartilage from the femoral head with either a scalpel blade, periosteal elevator, or osteotome. The processing instructions may dictate leaving the cartilage "on" when appropriate.
- D. Using a 3/8" drill bit, drill the cut end of the shaft approximately 5 cm. Thoroughly wash the interior of the intramedullary canal with the lavage system.
- E. Insert the intercalary fitting by screwing the threaded, tapered end into the cut end of the graft.
- F. Assemble the vacuum tubing by securing one end of the tubing to the nipple end of the intercalary fitting. Secure the other end of the tubing to the piston driven pump. Finally, secure another section of vacuum tubing to the other side of the piston pump.

- G. Pour approximately 4000 cc of a 1:100 dilution of the "Bone Cleaning Solution" into the sterile flushing vessel. The "Bone Cleaning Solution" is prepared by adding 4 cc of cleaning reagent to 3996 cc of sterile water. Refer to PRO-MS-136. Ensure the flushing vessel is clearly labeled as "Bone Cleaning Solution".

The temperature of the "Bone Cleaning Solution" solution must be between 37° and 44°C.

- H. Critical step: Place the "open" end of the second piece of vacuum tubing into a graduated Fleaker™ flask. Set the piston pump to "reverse" and set the flow rate controller to 50%. Turn on the pump and vacuum at least 500 cc of the "Bone Cleaning Solution" to waste. Record the vacuum times, the approximate volume of solution, and the flow rate controller setting on the "Tissue Processing Log Worksheet". Do not allow the level of fluid in the column to fall below the intercalary fitting.
- I. Critical step: Remove the "open" end of the second piece of vacuum tubing from the graduated Fleaker™ flask and place it into the sterile flushing vessel. Maintain the drive in the "reverse" position at 50%. Allow the "Bone Cleaning Solution" to recirculate for a minimum of 15 minutes. Record the temperature, exposure time, and flow rate controller setting on the "Tissue Processing Log Worksheet."
- J. Critical step: Decant the 1:100 dilution of the "Bone Cleaning Solution" and add approximately 4L of 3% hydrogen peroxide to the flushing vessel. Set the piston pump to "reverse" and set the flow rate controller to 50%. Turn on the pump and vacuum at least 500 cc of the 3% hydrogen peroxide solution to waste. Record vacuum time, approximate volume of discarded solution, and the flow rate controller setting on the "Tissue Processing Log Worksheet". Do not allow the level of fluid in the column to fall below the intercalary fitting.

- K. Critical step: Remove the "open" end of the second piece of vacuum tubing from the graduated Fleaker™ flask and place it into the sterile flushing vessel. Maintain the flow rate controller in the "reverse" position at 50%. Allow the hydrogen peroxide to recirculate for a minimum of 15 minutes. Ensure the flushing vessel is clearly labeled as "3% Hydrogen Peroxide". Record the exposure time and the flow rate controller setting on the "Tissue Processing Log Worksheet."
- L. Critical step: Decant the hydrogen peroxide and add approximately 3980 cc of sterile water and the entire reconstituted vials of Bacitracin and Polymyxin B to the flushing vessel. Refer to PRO-MS-010. Ensure the flushing vessel is clearly labeled as "Antibiotic." Set the piston pump to "reverse" and set the flow rate controller to 50%. Turn on the pump and vacuum at least 500 cc of antibiotic solution to waste. Record vacuum time, approximate volume of discarded solution, and the flow rate controller setting on the "Tissue Processing Log Worksheet". Do not allow the level of fluid in the column to fall below the intercalary fitting.

The temperature of the water/antibiotic solution must be between 37° and 44° C.

- M. Critical step: Remove the "open" end of the second piece of vacuum tubing from the graduated Fleaker™ flask and place it into the sterile flushing vessel. Maintain the drive in the "reverse" position at 50%. Allow the antibiotic solution to recirculate for a minimum of 15 minutes. Record the temperature of the antibiotic solution, exposure time, and flow rate controller setting on the "Tissue Processing Log Worksheet."

- N. Critical step: Decant the antibiotic solution and add approximately 4L of 70% isopropyl alcohol to the flushing vessel. Ensure the flushing vessel is clearly labeled as "70% IPA." Set the piston pump to "reverse" and set the flow rate controller to 50%. Turn on the pump and vacuum at least 500 cc of 70% IPA to waste. Record vacuum time, approximate volume of discarded solution, and the flow rate controller setting on the "Tissue Processing Log Worksheet". Do not allow the level of fluid in the column to fall below the intercalary fitting.
- O. Critical step: Remove the "open" end of the second piece of vacuum tubing from the graduated Fleaker™ flask and place it into the sterile flushing vessel. Maintain the drive in the "reverse" position at 50%. Allow the IPA to recirculate for a minimum of 15 minutes. Record the exposure time and the flow rate controller setting on the "Tissue Processing Log Worksheet."
- P. Critical step: Decant the 70% isopropyl alcohol and add 4L of sterile water to the flushing vessel. Ensure the flushing vessel is clearly labeled as "Sterile Water." Set the piston pump to "reverse" and set the flow rate controller to 50%. Turn on the pump and vacuum at least 500 cc of sterile water to waste. Record vacuum time, approximate volume of discarded solution, and the flow rate controller setting on the "Tissue Processing Log Worksheet". Do not allow the level of fluid in the column to fall below the intercalary fitting.
- The temperature of the sterile water must be between 37° and 44° C.
- Q. Critical step: Remove the "open" end of the second piece of vacuum tubing from the graduated Fleaker™ flask and place it into the sterile flushing vessel. Maintain the drive in the "reverse" position at 50%. Allow the water to recirculate for a minimum of 15 minutes. Record the temperature of the water, exposure time, and the flow rate controller setting on the "Tissue Processing Log Worksheet."
- R. Disconnect the tubing and remove the intercalary fitting from the graft.

S. Remove any remaining soft tissue if necessary and rinse again before culturing.

If the sterile wire wheel is used to remove the remaining soft tissue, ensure the Lucite™ capture box is surrounding the graft to minimize soft tissue discharge.

T. Assign the graft the appropriate identification number, record the measurements on the processing log worksheet (record the side (R/L), femoral head size, and total graft length). Culture the fashioned graft for bacterial contamination with sterile cotton-tipped applicators. Place one cotton-tipped applicator into a TGC tube, and one into a TSB tube, label with the graft identification number. Refer to PRO-MS-060.

U. The graft material is now ready for packaging and placement in the appropriate freezer. Refer to PRO-MS-064. The graft must be x-rayed if requested.